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A time-series study of sick building syndrome: chronic, biotoxin-associated illness from exposure to water-damaged buildings

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Abstract

The human health risk for chronic illnesses involving multiple body systems following inhalation exposure to the indoor environments of water-damaged buildings (WDBs) has remained poorly characterized and the subject of intense controversy. The current study assessed the hypothesis that exposure to the indoor environments of WDBs with visible microbial colonization was associated with illness. The study used a cross-sectional design with assessments at five time points, and the interventions of cholestyramine (CSM) therapy, exposure avoidance following therapy, and reexposure to the buildings after illness resolution. The methodological approach included oral administration of questionnaires, medical examinations, laboratory analyses, pulmonary function testing, and measurements of visual function. Of the 21 study volunteers, 19 completed assessment at each of the five time points. Data at Time Point 1 indicated multiple symptoms involving at least four organ systems in all study participants, a restrictive respiratory condition in four participants, and abnormally low visual contrast sensitivity (VCS) in 18 participants. Serum leptin levels were abnormally high and alpha melanocyte stimulating hormone (MSH) levels were abnormally low. Assessments at Time Point 2, following 2 weeks of CSM therapy, indicated a highly significant improvement in health status. Improvement was maintained at Time Point 3, which followed exposure avoidance without therapy. Reexposure to the WDBs resulted in illness reacquisition in all participants within 1 to 7 days. Following another round of CSM therapy, assessments at Time Point 5 indicated a highly significant improvement in health status. The group-mean number of symptoms decreased from 14.9±0.8 S.E.M. at Time Point 1 to 1.2±0.3 S.E.M., and the VCS deficit of approximately 50% at Time Point 1 was fully resolved. Leptin and MSH levels showed statistically significant improvement. The results indicated that CSM was an effective therapeutic agent, that VCS was a sensitive and specific indicator of neurologic function, and that illness involved systemic and hypothalamic processes. Although the results supported the general hypothesis that illness was associated with exposure to the WDBs, this conclusion was tempered by several study limitations. Exposure to specific agents was not demonstrated, study participants were not randomly selected, and double-blinding procedures were not used. Additional human and animal studies are needed to confirm this conclusion, investigate the role of complex mixtures of bacteria, fungi, mycotoxins, endotoxins, and antigens in illness causation, and characterize modes of action. Such data will improve the assessment of human health risk from chronic exposure to WDBs.

Keywords: Toxins; Fungi; Water-damaged indoor environments; Sick building syndrome; Visual contrast sensitivity; Cholestyramine; Leptin; Alpha melanocyte stimulating hormone

1. Introduction

The phrase "sick building syndrome" (SBS) has been used in reference to nonspecific health complaints thought to be associated with occupancy of certain buildings. The symptoms of SBS included any combination of sensory irritation, cough, wheezing, headache, cognitive disturbances, depression, light sensitivity, gastrointestinal distress, fatigue, weakness, pains, and other flu-like symptoms for which there have often been no objective signs [79,112,123]. The presence of symptoms was associated with exposure to specific indoor environments, and cases usually

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reported at least partial symptom resolution when removed from exposure. Occurrences of SBS were attributed to a variety of causes, including the emission of volatile organic compounds from building materials, pesticides, tobacco smoke, lighting, air exchange or circulation rates, and carbon monoxide, carbon dioxide, temperature, and humidity levels [141]. SBS complaints were tentatively attributed to microbial amplification, the visible colonization of microbes on surfaces in water-damaged buildings (WDBs), with increased frequency in recent years [2,3,47,61,73,74,83,107,124,131,140,144]. Evidence indicated that the indoor air contained complex mixtures of fungi, bacteria, mycotoxins, endotoxins, antigens, and biologically produced volatile organic compounds to which building occupants were exposed through inhalation [11,32,51,64,93,94,109,131,134,150,152–154]. There was general acceptance of the hypothesis that chronic exposure to the air in WDBs is associated with allergic and irritant effects on pulmonary function [31]. Ample evidence indicated that asthma and hypersensitivity pneumonitis in children [10,13,67,88,102,117] and adults [6,15,50,52, 62,65,95,121,150,159,161] were associated with atopy and inflammation triggered by exposure to biologic contaminants in indoor air. Additional evidence indicated that dampness and fungi in schools were associated with respiratory distress in children [18,42,46,98,99,108,110, 132,146], although some evidence indicated that toxic, rather than allergic, processes were involved [87,147,148]. Fungal mycotoxins were the suspected cause of pulmonary hemorrhage/hemosiderosis in infants [49,59,60, 89,114,122,149,154,158]. *Stachybotrys chartarum* (a.k.a. *S. atra*) strains with high hemolytic activity were isolated from the indoor air and the bronchoalveolar fluid of children with pulmonary hemorrhage [54,153,154]. The Centers for Disease Control and Prevention (CDC) initially confirmed [27,28], but later questioned, the association of mycotoxin inhalation and infant hemosiderosis [30]. Even more controversial [31] was the hypothesis that toxins inhaled in WDBs are associated with a multiple-system syndrome [45,69,92,143], although the need for research that better characterized this potential human health risk has been well recognized [1–3,20,33,37,38,47,51,58,61,75,83,100,107,124,129,131, 140,145]. The scientific literature on the association between multiple-system symptoms and inhalation exposure to toxigenic microbes in WDBs is reviewed below, and a new study is described that attempted to overcome some of the methodological limitations identified in the previous studies.

Previous case reports and research studies have associated damp building environments and indoor microbial amplification with a multiple-system syndrome. However, the associations have generally been viewed as weak due to several methodological limitations. An early report by Croft et al. [40] described multiple-system symptoms in individuals living in a water-damaged home where *S. chartarum* was identified. Repeated medical evaluations, not well described in the report, failed to detect medical or laboratory abnormalities. The diagnosis of mycotoxicosis was made following trichothecene mycotoxin extraction from fungal swab samples, some symptom subsidence after removal from the home, and the lack of symptom recurrence during reoccupancy of the remediated building. Criticisms concerning the lack of exposure demonstration [145] were addressed in a recent report by Croft et al. [41]. Novel procedures were used to extract trichothecenes from the urine of four cases with multiple-system symptoms and exposure to buildings exhibiting microbial colonization. Extracted mycotoxins injected into weanling rats caused severe degeneration in the central nervous system and lung scarring. Although supportive of an association, methodological limitations inherent in case reports precluded firm establishment of an association.

Many studies have demonstrated the presence of *Stachybotrys* [5,11,72] and other fungal genera, including *Aspergillus*, *Penicillium*, *Cladosporium*, *Chaetomium*, *Ulocladium*, and *Alternaria* [35,55,72,109,134] in dust, on building materials and in the air of WDBs. Only weak associations were observed between fungal species identified on surfaces and in air [35], presumably because the time course of fungal-spore release to air is irregular. The rate of spore release to air is influenced by many factors, including microbial genera, surface hydrologic conditions, air currents and velocities, and interspecies competition [108]. Additional studies identified mycotoxins in dust or fungal samples [5,55,72,118,139,152,153]. The search for biomarkers of fungal exposure showed that much of the general population has IgG and/or IgE antibodies to fungi, but these markers were not diagnostic of disease, and have not discriminated between occupants of contaminated and uncontaminated buildings [1,11,38,150]. Total spore counts [132] and levels of β(1→3)Glucan, a marker of biomass, showed statistically significant correlations with indoor pollutants, such as endotoxins, allergens, and fungi [66]. Although most of these studies did not examine relationships to the symptoms of building occupants, a literature review reported associations between β(1→3)Glucan levels, symptom prevalence, and measures of inflammation [131]. Both in vitro [76,84,101,115] and rodent [93,94,119,120,125,156] studies have demonstrated the ability of bacteria and fungi commonly found in WDBs or their toxins to elicit cytotoxicity and an inflammatory response, often in dose–response fashion [84,93,94,101,115,119,120]. Inflammatory responses to bacteria most often involved tumor necrosis factor alpha, interleukin 6, interleukin 1 beta, and nitric oxide [76,84,93,115], whereas fungi or their metabolites most often induced tumor necrosis factor alpha, interleukin 6, and interleukin 2 [84,94,101,156]. The observations that β(1→3)Glucan levels were correlated with symptom prevalence and inflammation, and that many bacteria and fungi elicited similar proinflammatory cytokine responses indicated that SBS in occupants of WDBs may be induced by the

total biological load in inhaled air through a combination of toxic and immunologic processes.

Several studies have investigated complaints of SBS in populations occupying WDBs, but none have provided evidence sufficient to conclude that illness was firmly associated with exposure to biological agents. Johanning et al. [91] collected data using a self-administered symptom questionnaire and laboratory analyses from a cohort working in a water-damaged and fungal-infested building, and a control group. The prevalence of multiple-system symptoms differed between groups, and was positively correlated to the duration of employment in the exposed group. A small but statistically significant group difference in a biomarker of the immune system was also observed. The main shortcomings of the study were the lack of (1) data concerning fungal exposure in the control group; (2) evidence eliminating potentially confounding factors, such as preexisting illnesses and other exposures; (3) control for potential bias in the self-administered questionnaire data; (4) statistical control for multiple comparisons; and (5) a robust, objective finding. Similar shortcomings were present in another case-control study that identified large differences in questionnaire-reported symptoms between occupants of three water-damaged and fungal-contaminated buildings, and workers from two control buildings [77]. Neuropsychological tests provided objective evidence of effects in two studies by demonstrating cognitive and emotional profiles in exposed cases similar to those from patients with mild traumatic brain injury [9,70]. However, the other limitations discussed above were not addressed.

Two cross-sectional studies with intervention overcame several of the limitation discussed above, but lacked objective indicators of effect [53,144]. Ebbehoj et al. [53] reported that visible inspection and surface sampling in an office building identified evidence of high moisture and microbial growth with *Trichoderma* and *Phoma* fungi as the dominant genera. Mean multiple-system symptom scores in study participants were 66%, and variability in peak expiratory flow rate was 20%. Following building remediation, no visible evidence of microbial contamination was present. However, symptom scores decreased only to 33%, and sampling revealed the continued presence of microbes. Following extensive cleaning, microbial levels decreased, and symptom scores declined to 4%. Variability in peak flow rate dropped to 15%. The intervention and time-series components of this study added power to the experimental design by enabling study participants to serve as their own controls, thereby controlling many potentially confounding factors. The main study deficiency was the lack of a robust and objective indicator to support the reports of symptoms involving nonrespiratory systems. A health assessment was conducted by the National Institute of Occupational Safety and Health after workers in a water-damaged office building complained of health effects [144]. Moderate to high levels of *Aspergillus* sp., *Penicillium* sp., and bacteria were found in bulk and surface samples. *Stachybotrys* sp. were identified in only 5% of samples cultured in Czapek agar. The prevalence of multiple-system symptoms, primarily neurologic and respiratory, was significantly higher prior to removal from the building than 6 months after removal. The lack of objective indicators of effect in these studies continued to prevent firm establishment of an association between indoor microbial exposure and adverse health effects.

An investigation of chronic respiratory and flu-like symptoms among construction workers at the Denver International Airport also lacked a robust, objective indicator of illness [57]. The rate of cases, defined as having at least two lower respiratory complaints and one systemic symptom, was 34% among randomly selected workers and 2% in controls who had not worked at the airport. Length of employment was a statistically significant risk factor for illness. An exposure assessment identified alkaline dust in the work environment of cases, and widely distributed airborne *Penicillium* in the airport. In addition to the lack of an objective indicator for a systemic effect, the ability to draw definitive conclusions from the study was hampered by the lack of exclusion for potentially confounding factors, and the unavailability of information about alkaline dust and *Penicillium* exposures among controls.

Several other cross-sectional studies supported the hypothesis that exposure to microbes in excessively damp buildings is associated with a multiple-system syndrome, but lacked the objective indication of effect in a non-pulmonary system, and the experimental-design power needed to reach the level of scientific certainty. A British study reported positive associations between SBS symptom prevalence rates and the levels of viable airborne fungi and bacteria within office buildings [74]. A Swedish study surveyed occupants in more than 14,000 residences in 609 multifamily buildings, and yielded a 77% response rate [56]. Over 28% of respondents reported at least one of four signs of dampness, and all dampness indicators were associated with significantly increased rates of multiple-system symptoms after statistical adjustments for several socio-demographic factors. In dwellings with all four dampness indicators, highly elevated odds ratios were reported for ocular, nasal, throat, and dermal symptoms, as well as for cough, headache, and tiredness. Results from a one-year study of 98 workers in four Bostonian office buildings were recently reported [32]. Concurrent surface sampling and questionnaire administration at 6-week intervals positively associated total fungal concentrations in floor or chair dust with reports of eye irritation, a group of nonspecific symptoms, asthma, and upper respiratory symptoms. However, fungal concentrations were also associated with low job satisfaction, office crowding, and lack of office cleanliness. Another cross-sectional study observed relationships between SBS symptoms and prior physician diagnoses of dust or mold allergy [104]. A statistical cluster analysis defined problematic areas in which potentially etiologic microbes were identified.

Although these studies associated indoor microbial contamination with a multiple-system syndrome, scientific certainty required that several study limitations be overcome. First, potential study participants should be screened for exclusion due to confounding factors using a standard tool to assess medical and lifestyle history, as well as occupational, residential, and avocational exposures to toxic substances. Second, the study should include a comprehensive medical evaluation and differential diagnosis techniques to identify illnesses unrelated to building exposure, but capable of causing SBS symptoms. Third, the potential for bias and imprecision in self-reported symptoms, particularly in self-administered questionnaires, must be reduced. An interview of study participants by a single, highly trained and practiced medical researcher using a standard set of questions designed to assess symptoms and illness duration is needed to reduce bias and increase precision. Fourth, because indoor exposures to microbes are generally acknowledged to have the potential to affect the respiratory system, but are only hypothesized to affect other systems, a robust indicator of effect in another system is needed. Fifth, better control of unidentified but potentially confounding factors in study participants is needed. One approach is to use an experimental design that allows participants to serve as their own controls, as in the time series with intervention design used by Ebbehoj et al. [53] and described above. Sixth, a still more powerful experimental design, often used in animal studies, would include illness remission during intervention, followed by illness reacquisition upon reexposure without therapy. This approach has not been feasible due to the lack of an effective and rapid therapeutic intervention. Use of these methodological and design elements would improve the ability of a study to firmly associate SBS with exposure to biologic agents.

Another critical methodological consideration is exposure assessment. Microbial growth on surfaces in WDBs is often a complex mixture of fungal and bacterial species. The studies reviewed above demonstrated a wide variety of predominant organisms in WDBs. Although many species were known to be toxigenic, it is generally acknowledged that a comprehensive list of bioactive components, including mycotoxins, endotoxins, and antigens, does not exist at this time. It is impossible, therefore, to quantify all bioactive components on building surfaces, in building air, or in biological tissue. Air sampling is particularly problematic because the release of spores to air is irregular, as discussed above [111]. The relationship between exposure to mixtures and health effects is obscured by the potential for synergistic interactions between bioactive components, such as the synergistic effect of *Streptomyces californicus* and *S. chartarum* or fungal toxins on the production of proinflammatory cytokines [85]. Because of these limitations, it is inappropriate to conclude that exposure levels to any single species or component, or even groups, is too low for exposure to that environment to cause SBS symptoms in building occupants. In many studies of illness in occupants of WDBs, an appropriate hypothesis is that chronic exposure to the water-damaged environment is associated with illness. Exposure assessment should include visual observation of the environment, and characterization of water-damaged surfaces and microbial amplification. Identification of organisms and components should be done to the extent practical. Confirmation of the hypothesis should be based on the experimental design elements discussed above. Animal studies will be needed to test additional hypotheses concerning microbial causation of illness, and to assess the relative potency of individual organisms, components, and mixtures.

Pilot data indicating that the paradigm of biotoxin-associated illness may generalize to SBS associated with WDBs [78] provided impetus for the current study. Both acute and chronic biotoxin-associated illnesses were previously ascribed to cases diagnosed with Possible Estuary Associated Syndrom (PEAS; [135,137]), a condition with onset following exposure to estuaries where the toxigenic dinoflagellates, *Pfiesteria* sp. [29], were associated with massive fish kills [21] and human illness [68,71]. The cases were characterized by multiple-system symptoms, a deficit in visual contrast sensitivity (VCS) indicating neurologic impairment [81], elevated levels of proinflammatory cytokines, and the absence of alternative explanations of illness [135,137]. Cases were treated with cholestyramine (CSM; Questran), a nonabsorbable polymer with anion exchange capacity, known to eliminate a variety of toxins and drugs by interrupting their enterohepatic recirculation with bile [8,94]. Symptoms resolved as VCS normalized during 2 weeks of CSM therapy in both acute and chronic cases [135,137]. A double-blinded, placebo-controlled, crossover clinical trial demonstrated that symptom resolution and VCS recovery occurred only during CSM therapy [135]. PEAS cases showed no relapse without reexposed to *Pfiesteria*-inhabited estuaries. When reexposed, recovery again promptly followed CSM therapy [137]. VCS measurements provided an objective indication of neurologic function in illness acquisition and recovery in this condition otherwise characterized only by nonspecific symptoms. The efficacy of CSM therapy supported the hypothesis of biotoxin-induced illness. Pilot data collected from SBS cases occupying WDBs demonstrated relationships between multiple-system symptoms, VCS alterations, and response to CSM therapy similar to those observed in PEAS cases [78], indicating that biotoxin-associated illness may underlie this condition.

The current study addressed the general hypothesis that SBS is associated with exposure to WDBs. Specific hypotheses concerning symptoms, VCS, and hormones were tested to assess the validity of the general hypothesis. The cross-sectional study design included environmental assessments, medical and laboratory evaluations of partic-

ipants' health status at five time points, and the interventions of CSM therapy, removal from exposure and reexposure. The study design and procedures were selected to address many of the limitations identified in previous studies.

2. Methods

2.1. Study hypotheses

The general hypothesis of the study was that SBS is associated with chronic exposure to WDBs. Confirmatory hypotheses were that the group-average number of symptoms and VCS scores would (1) improve with CSM therapy, (2) remain stable during exposure avoidance without therapy, (3) worsen with reexposure, and (4) again improve with resumption of CSM therapy. Additional hypotheses concerned blood levels of the hormones leptin and alpha melanocyte stimulating hormone (MSH). The hypotheses were that leptin levels would decrease and MSH levels would increase following CSM therapy.

2.2. Building identification

Each of the five occupational buildings was identified as a possible SBS building when one employee from each of the buildings presented at a private clinic in Pocomoke City, MD, seeking medical attention (the index cases). The oral administration of questionnaires on symptoms, medical history, occupational history, and life style factors revealed chronic health symptoms in each patient. Each building was described as having a history of water damage (Table 1). Site investigations were conducted at each building between September 2000 and June 2002. Four of the buildings were visually inspected by licensed industrial hygienists who specialized in indoor environment investigations. Evidence of water damage from one or more sources was observed in each building as described in Table 1. Microbial amplification was observed in the areas of water damage, and tape-lift or bulk samples were collected from each site. Samples were sealed in air-tight containers and examined microscopically to identify fungal genera and species. The fifth building was inspected by a supervisor of employees in the building. The supervisor noted several areas of water intrusion and microbial colonization. The supervisor followed a protocol provided by P & K Microbiology, (Cherry Hill, NJ) for sample collection. The samples were shipped to P & K Microbiology for microbial analysis. In all five buildings, qualitative microscopic analyses identified one or more genera of fungi colonizing water-damaged surfaces in each building (Table 1). Analyses of mycotoxins, bacteria, endotoxins, and antigens were not undertaken.

2.3. Study participant recruitment and screening

The index case from each building was examined by the first author, a licensed medical doctor. All five index cases were diagnosed by the physician as having possible SBS due to chronic exposure to water-damaged indoor environments. The criteria for possible SBS diagnosis were derived from criteria defined by the Centers for Disease Control and Prevention for diagnosis of PEAS [29]. The criteria were:

(1) exposure potential—chronic, occupational exposure to the indoor environment of a building showing evidence of water damage and microbial amplification;
(2) multiple system symptoms—symptoms in at least four of the eight organ systems listed in Table 2 that persisted for >2 weeks;
(3) absence of confounders—the clinician was unable to identify other possible causes of illness using differential diagnosis techniques.

The study protocol, questionnaires, and consent form were approved by an authorized internal review board, Copernicus IRB, Cary, NC. The index case from each building informed other employees in the building of the

Table 1
Building description, microbial contamination and study participant selection

	Building use	Date inspected	Water leakage	Predominant species	# Screened	# SBS[a]	# Enrolled
Building 1	Court house	9/00	Plumbing, basement	*Aspergillus ustus*, *Penicillium aurantiogriseum*	6	6	3
Building 2	Penitentiary	1/02	Roof	*Acremonium* sp.	5	5	5
Building 3	Social services	3/02	Roof, plumbing	*Aspergillus niger Penicillium* sp., *Stachybotrys chartarum*	15	13	5
Building 4	Multiple services	4/02	Plumbing	*Stachybotrys chartarum*, *Cladosporium* sp.	20	13	4
Building 5	Police department	6/02	Flooding	*Aspergillus* sp., *Acremonium* sp., *Stachybotrys chartarum*	4	4	4
Totals					50	41	21

[a] The screening results indicated possible SBS.

Table 2
Percent of participants with symptoms, by organ system and time point, and previously published control values [135]

Time Point/Control	General		Muscle			Unique	Eye			Respiratory			Gastrointestinal		General neurologic				Central nervous system					
	Fatigue	Weakness	Aches	Cramps	Unusual pains	Joint pains	HA	Light Sensitive	Red eye	Blurred vision	Tearing	Sinus congestion	Cough	SOB	Abdominal pain	Diarrhea	Numbness	Tingling	Metal taste	Vertigo	Memory	Concentration	Confusion	Learning	Word find	Disoriented
1	100	84	95	63	42	84	84	89	63	63	63	74	53	74	37	21	37	47	47	42	68	53	26	37	11	11
2	32	5	11	0	0	21	32	11	0	0	11	0	11	5	5	0	5	0	0	0	16	0	0	0	0	0
3	16	5	5	0	0	21	11	5	0	0	5	5	0	5	0	0	0	0	0	0	5	0	5	0	0	0
4	95	63	58	26	16	68	84	63	21	58	11	42	47	47	16	11	5	16	5	21	58	21	26	16	5	5
5	26	5	5	0	0	16	16	0	0	0	0	5	16	11	5	0	0	5	0	0	16	0	0	0	0	0
C	2	0	1	0	NA	NA	2	1	0	NA	NA	2	3	2	2	0	NA	NA	NA	1	2	0	0	NA	NA	NA

General system symptoms: fatigue, weakness.
Muscle system symptoms: aches, cramps, unusual pains, joint pains.
Unique system symptoms: headache.
Eye system symptoms: light sensitivity, red eyes, blurred vision, tearing.
Respiratory system symptoms: sinus congestion, cough, shortness of breath.
Gastrointestinal system symptoms: abdominal pain, diarrhea.
General neurologic system symptoms: numbness, tingling, metallic taste, vertigo.
Central nervous system symptoms: memory loss, concentration difficulty, confusion, learning difficulty, word finding difficulty, disoriented.

proposed study, and invited them to the clinic for screening and possible inclusion in the study. Fifty employees who visited the clinic were examined by the first author. Data on medical history, symptoms, and potentially confounding factors were collected through oral administration of questionnaires by the physician. The employees were also questioned about the study buildings' histories, including water damage, visual detection of microbial colonies, olfactory detection of odors including musty smell, building usages, upkeep, remediation and renovation, and the presence of possible chemical contaminants. The potential study participants also received a physical examination during screening. The results indicated that 41 employees were possible SBS cases (Table 1), none of whom were involved in litigation or worker's compensation claims. The cases reported little or no relief from symptoms during vacations or other time periods when away from the buildings. Possible cases were offered treatment outside of the study if they did not meet the study inclusion criteria. Employees were excluded from study participation if they had a history of possible biotoxin-associated illness from sources other than the study buildings, such as exposure to water-damaged residences, to fresh water cyanobacteria, to tick bite illness (e.g., Lyme disease), to *Pfiesteria*-inhabited estuaries, to contaminated seafood (e.g., *Ciguatera* seafood poisoning), and to poisonous bites (e.g., brown recluse spider). Other exclusion criteria included excessive exposure to solvents, airborne metals or hydrocarbons, chronic alcoholism with indication of liver disease, neurologic disease, untreated asthma, a diagnosis of hypersensitivity pneumonitis prior to working in a study building, current pregnancy or breast feeding, and age <18 years. Employees meeting the inclusion criteria were informed about study details. Treatment was offered through study participation or outside of the study. Twenty-one employees volunteered for the study and signed informed consent forms (Table 3). Nineteen of the study participants completed assessments at all five time points (Table 4; mean age=46.4 years, age range=29–62 years, 4 males and 15 females). One participant withdrew from the study due to disabling illness experienced during the building reentry phase of the study. Another participant was disqualified because of building remediation prior to the reentry phase of the study.

2.4. Study design

A cross-section of building occupants was examined in a study using an ABAB design. The study design included a five-point time series of medical assessments and three types of interventions, CSM therapy, exposure avoidance, and reexposure. In addition to the oral administration of questionnaires and conduction of physical examinations during screening, study participants underwent visual and pulmonary function testing and blood draws during study

Table 3
Study participants

Building #	Participant #	Age (years)	Gender	Ethnicity	Illness duration (months)	Previous diagnoses	Smoker	Days to relapse at TP4	Weeks to complete study
1	1	52	F	AA	48	Ro, CFS, FM	N	2	5
	2	30	M	C	12	Si, CFS	N	2	5
	3	50	F	C	24	Tinnitius, Ro, Dp	N	3	7
2	4	43	F	C	12	Myasthenia, lupus, FM	Y	3 (×2)	12
	5	50	F	C	12	Detrusor dyssynergy, DJD, Dp	N	5	7
	6	46	F	AA	1	Thalessemia, Al	N	3	6
	7	40	F	C	18	CPS, migraine, FM	N	2	5
	8	50	F	C	24	Dp, Si, Al	N	3	6
3	9	59	F	C	120	Al, edema, RLS	N	4	5
	10	49	F	C	120	CFS, D	N	2	–[a]
	11	48	F	C	36	Anemia, FM	N	4	8
	12	58	F	C	120	DJD, Dp	N	3	5
	13	32	F	AA	18	Reflux, Al	N	2	5
4	14	62	F	C	24	Arthritis, DJD	N	2	5
	15	54	M	C	8	AODM, HBP, FM	N	7	7
	16	49	F	C	48	CTS, AODM, CFS	2ndH	2	5
	17	44	F	C	24	FM, ADHD	N	2	6
5	18	41	M	C	24	Impotence	N	1	5
	19	44	M	C	24	Al, Ob	N	3	6
	20	29	F	C	24	Si, FM	N	1	5
	21	47	F	C	24	Ob, Dp	Y, 2ndH	–[b]	–[b]

TP4=Time Point 4, M=male, F=female, C=caucasian, AA=African American, Y=yes, N=no, 2ndH=secondhand smoke at home.
(×2)=Participant was reexposed twice and became ill within 3 days both times; repeated Time Points 4 and 5.
Al=allergy, Dp=depression, Ob=obesity, Si=sinusitis, Ro=rosacea, CSF=chronic fatigue syndrome, FM=fibromyalgia, ADHD=attention deficit, hyperactivity disorder, DJD=degenerative joint disease, AODM=adult onset diabetes mellitus, CPS=chronic pain syndrome, RLS=restless leg syndrome, HBP=high blood pressure, CTS=carpal tunnel syndrome.
[a] Withdrew from study due to illness.
[b] Building was remediated prior to reexposure phase of study.

Time Point 1. The questionnaires, physical examination, vision testing, and blood analyses were repeated at each of the time points with the exception that blood was not drawn during Time Point 3. Pulmonary function measurements were repeated only at Time Point 5. The study interventions were initiated immediately after medical assessments at each of the five time points, and scheduled as:

Time Point 1—began CSM therapy for 2 weeks;
Time Point 2—ended CSM therapy and began avoidance of the study buildings for at least 7 days;
Time Point 3—returned to work in the study buildings without therapy for 1–7 days;
Time Point 4—again began CSM therapy for 2 week;
Time Point 5—ended CSM therapy.

Cholestyramine therapy was prescribed by the physician and administered by the study participants. The dose schedule was 9 g of CSM dissolved in 6 oz of apple juice or water administered orally, four times per day. Participants were questioned concerning compliance with therapy at Time Point 2–5 medical assessments. All participants reported taking the prescribed dose of CSM three to four times per day, and were judged as compliant. All patients reported compliance with building avoidance between Time Points 2 and 3, and with reoccupancy of buildings between Time Points 3 and 4.

Participants were advised at the end of the study to initiate prophylactic CSM therapy if they chose to return to the WDB environment prior to successful building remediation.

2.5. Blood analyses

Laboratory analyses of blood samples included a complete blood count, comprehensive metabolic profiles, and measurement of leptin and MSH levels. The latter two measurements were not initiated until after assessment of the three participants from Building One was completed. MSH was measured only at Time Points 1 and 5. Blood analyses were performed by LabCorp, or Esoterix, both of which are CLIA approved, high-complexity laboratory facilities. Normal ranges for leptin in men, 1–5.8 ng/ml serum, and in women, 5–18.3 ng/ml serum, unadjusted for body mass, were reported by Esoterix. LabCorp reported a normal MSH range of 35–81 pg/ml serum.

2.6. Pulmonary function testing

Pulmonary function was assessed in all patients using a Micro Medical spirometer. Forced expiratory volume in 1 s

Table 4
Vision, symptoms, leptin, and MSH for study participant assessed at all 5 time points

P#	VT1	VT2	VT3	VT4	VT5	ST1	ST2	ST3	ST4	ST5	LT1	LT2	LT4	LT5	MSH[a]	MSH[b]
1	48.5/11.5	86.5/43	128/43	54.5/15	96/50	14	1	1	9	0	NA	NA	NA	NA	NA	NA
2	180/102.5	180/120	180/102.5	77/60	180/102.5	8	1	2	7	0	NA	NA	NA	NA	NA	NA
3	77/51.5	154/85	154/102.5	77/43	128/72.5	15	0	0	7	0	NA	NA	NA	NA	NA	NA
4	90/30	128/60	128/60	45/11	128/85	11	1	1	10	2	30	37	45	23	7.5	21
5	77/30	128/60	154/90	128/45	154/120	18	2	1	9	1	49	NA	57	39	7.5	NA
6	54.5/18.5	96/51.5	128/72.5	64/51.5	128/60	15	2	1	8	0	46	NA	NA	22	22	46
7	40/25.5	96/25.5	109/22.5	64/15	109/36.5	17	4	2	11	4	17	NA	NA	7	7.5	NA
8	54.5/30	128/60	128/36.5	67.5/18.5	128/43	18	4	3	11	3	58	58	NA	45	7.5	19
9	39/7.5	77/22	90/26	48.5/18.5	109/30	17	2	2	10	1	49	25	38	22	35	NA
11	64/30	128/60	128/60	128/43	180/60	15	0	0	7	0	15	6	16	8	18	NA
12	19.5/0	67.5/7.5	64/15	48.5/11	54.5/4	17	2	1	10	3	83	NA	NA	25	16	21
13	64/30	128/72.5	128/85	64/43	135/72.5	19	2	1	11	2	86	57	84	57	27	37
14	64/22	64/30	64/43	33/22	90/60	13	1	0	8	1	43	NA	NA	35	7.5	NA
15	64/29	180/72.5	180/120	109/41	180/120	13	2	0	15	1	21	11	19	8	7.5	18
16	122/26	109/45	128/72.5	77/45	154/72.5	19	0	0	11	2	25	12	32	25	7.5	22
17	77/26	154/85	154/85	109/72.5	180/85	15	3	1	9	1	46	40	48	34	7.5	20
18	28/13	154/51.5	128/51.5	77/26	128/51.5	6	0	0	7	0	7	NA	NA	4	7.5	15
19	77/45	90/36.5	128/85	77/45	128/72.5	20	2	0	6	0	39	NA	NA	22	7.5	61
20	109/37.5	128/51.5	154/64	77/51.5	154/120	13	2	0	7	1	16	NA	NA	8	7.5	NA
M	71/30	120/55	129/65	75/36	134/69	14.9	1.6	0.8	9.1	1.2	39.4	30.8	42.4	24.0	12.5	28.0
SE	8.4/4.9	8.0/6.0	7.3/6.8	6.1/4.1	7.8/7.2	0.8	0.3	0.2	0.5	0.3	5.8	7.2	7.7	3.8	7.7	15.1

P#=participant number, M=mean, S.E.=standard error of the mean, NA=data not available.
V=mean VCS scores for two eyes at rows D and E (spatial frequencies 6 and 9 cycles per degree of visual arc). T1-5=Assessment Time Points 1, 2, 3, 4, and 5.
S=number of symptoms. L=leptin concentration. leptin levels were not measured at T3. Esoterix reported normal ranges of 1–5.8 ng/ml serum in men and 5.0–18.3 ng/ml serum in women. Missing data in participants 4–20 resulted from the lack of fasting by participants prior to assessments.
[a] Alpha melanocyte stimulating hormone levels measured at T1, values below the detection level of 15 are reported as 7.5. LabCorp, reported a normal range of 35–81 pg/ml serum for men and women.
[b] Alpha melanocyte stimulating hormone levels measured at T5. Missing data in participants 4–20 resulted from manufacturer supply shortages of aprotinin (Trasylol), a proteinase inhibitor, that is added to the chilled plasma tube after phlebotomy.

(FEV-1) and forced vital capacity (FVC) were measured. Pulmonary function in each participant was categorized according to the criteria:

Normal: FEV-1/FVC×100>80; FVC>80;
Obstructive (e.g., asthma): FEV-1/FVC×100<80; FVC>80;
Restrictive (e.g., hypersensitivity pneumonitis): FEV-1/FVC×100>80; FVC<80;
Obstructive and Restrictive: FEV-1/FVC×100<80; FVC<80.

2.7. Visual function testing

Near-point visual acuity and near-point VCS were measured using modified, forced-choice procedures previously described in detail [80,135,137]. Briefly, the acuity (MIS Pocket Vision Guide, © 1997 MIS) and VCS (Functional Acuity Contrast Test, F.A.C.T. 101; Stereo Optical, Chicago, IL, a Gerber–Coburn) test were administered monocularly to each eye under standard daylight spectrum illumination with normal office background illumination. Photometric measurements indicated a luminance of approximately 70 ft-L on the test card surfaces. All participants who used corrective lenses for near-point viewing wore them during vision testing. The a priori criterion for analysis of VCS data, that the eye has a Snellen Distance Equivalent visual acuity sore of 20:50 or better, was met by both eyes in 16 of 19 participants that completed the study; the acuity criterion was met by only one eye in participants 4, 11, and 14 (Table 4). This criterion avoided confounding of VCS scores as indicators of neurologic function by excessive optical-refraction error. The units of analysis of group-mean VCS scores were the mean scores of the participant's two eyes at each spatial frequency when both eyes met the acuity criterion. When only one of a participant's eyes met the acuity criterion, that eye's VCS scores were the units of analysis in group-mean assessments.

Assessments of VCS scores in individuals were based on criteria applied to the mid-three spatial frequencies (rows B, C, and D, 3.0, 6.0 and 12.0 cycles/degree of visual arc, respectively). Criteria for normal VCS were:

if VCS at row B=160 (stimulus ID #9), then VCS at row C≥128 (stimulus ID #8) and row D≥43 (stimulus ID #6); or
if VCS at row B<160 (stimulus ID #9), then VCS at row C≥90 (stimulus ID #7) and row D≥43 (stimulus ID #6).

If either eye of a participant failed to meet the criteria for normal VCS, then VCS in that participant was classified as abnormal.

2.8. Statistical analyses

All statistical analyses tested a two-tailed hypothesis of no difference at a significance level of alpha=0.05. The statistical procedure used to analyze the VCS data was multivariate analysis of variance for repeated measures (MANOVA). The VCS units of analysis were considered to be repeated measures because one unit for each of the five spatial frequencies was collected from each study participant in the same order at each of the five time points. The five VCS units collected from each participant at one time point were compared to the five VCS units collected at another time point in one analysis. This procedure was repeated for all 10 possible parings of data collected at the five time points. Bonferroni adjustments were made to all p-values to reduce the possibility of false-positive results due to the multiplicity of comparisons.

Three hypotheses were tested in each of the 10 MANOVA analyses of the VCS data. The first null hypothesis was that there was no difference between the data collected at the two time points. The analysis essentially averaged the five group-mean VCS units collected at each of two time points, and determined whether or not the two averages were statistically significantly different. The second null hypothesis was that there was no difference between the VCS units collected at each of the five spatial frequencies. This analysis averaged the VCS units from both time points at each of the five spatial frequencies, and determined whether or not there was a statistically significant difference among the five averages. This hypothesis should always be rejected because VCS scores are known to vary with spatial frequency. The third hypothesis was that there was no interaction effect between the study time points and the VCS spatial frequencies. This test essentially determined whether or not the VCS spatial-frequency profiles from the two study time points were parallel. Rejection of the hypothesis indicated that there was a change in relative sensitivity to the spatial frequencies between the time points.

The same statistical approach was used to assess differences between time points in symptom, leptin, and MSH levels. Each of these variables was measured only once at each study time point. In this case, the MANOVA analyses reduced to paired t-tests that were done for comparisons at each possible pair of times. Only pairs with data at both times were used in the comparisons. Bonferroni adjustments were made to symptom, leptin, and MSH p-values to control for the multiplicity of comparisons.

3. Results

3.1. Building descriptions

All five study buildings had visible evidence of water damage and microbial amplification (Table 1). Plumbing leaks were identified in three buildings, two buildings showed evidence of water intrusion in or around the roof, one building had periodic water intrusion through basement walls, and the ground floor of one building in a low-lying area was flooded periodically. Qualitative laboratory analyses of tape lift or bulk samples revealed multiple sites of fungal colonies in all study buildings. Predominant genera and species were *Aspergillus* sp. in three buildings, *S. chartarum* in three buildings, *Penicillium* sp. in two buildings, *Acremonium* sp. in two buildings, and *Cladosporium* sp. in one building (Table 1).

3.2. Participant demographics

Twenty-one workers from the five study buildings volunteered for study participation (Table 1). Participants ranged in age from 29 to 62 years, 4 were male and 17 were female, 3 were of African American ethnicity and 18 were Caucasian (Table 3). Illness duration ranged from 1 to 120 months, and the participants had previous diagnoses from physicians unaffiliated with the study for a variety of chronic health conditions (Table 3). Smoking was not permitted in any of the study buildings. Two participants were cigarette smokers, one of those also received second-hand smoke at home, and one nonsmoker received second-hand smoke at home (Table 3). All participants reported that previous extended periods without exposure to their office buildings had produced little or no relief from symptoms. Nineteen participants completed the study by receiving all study interventions and assessments at all five study time points. The time required to complete the study ranged from 5 to 12 weeks (Table 3).

3.3. Symptom, vision, physical, and laboratory results—Time Point 1 assessment

The 19 participants who completed the study averaged 14.9 symptoms out of 26 at Time Point 1 (Table 4). All participants had symptoms from at least four of the eight organ systems assessed, and the range of the number of symptoms was 6–20 (Table 4). The percentages of participants that reported each symptom are shown in Table 2. Symptom prevalence in the participants at Time Point 1 greatly exceeded that reported by 87 patients who served as controls in a previous study [135]. Over 60% of the study participants reported fatigue, muscle aches, sensitivity to bright light, weakness, joint pain, headache, sinus congestion, shortness of breath, memory loss, muscle cramps, red eyes, blurred vision, and tearing at Time Point 1. Although group-mean Snellen visual-acuity scores were normal (left eye=20:21.6; right eye=20:23.4), group-mean VCS scores of the participants were sharply reduced relative to the previously published control values [135] (Fig. 1). The VCS deficits were largest at the mid-to-higher spatial frequencies. VCS scores at 6 and 12 cycles/per degree of visual arc are shown for each of the 19 participants in Table

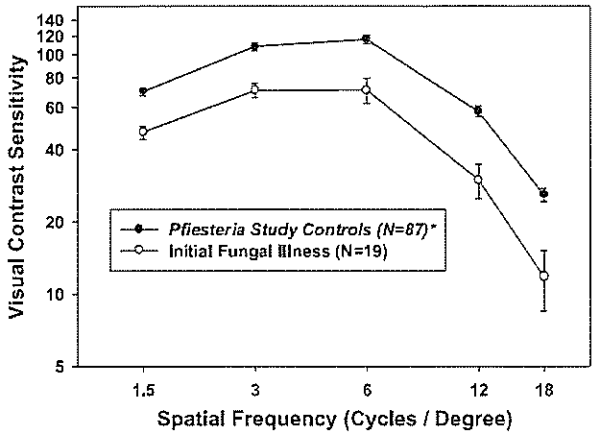

Fig. 1. Group-mean VCS scores (±S.E.M.) for SBS cases (mean age=46.4 years, age range=29–62 years) at Time Point 1, and for a previously published control group (mean age=45.1 years, age range=22–72 years) that did not have biotoxin-associated illness. Although the two groups did not differ in Snellen visual acuity, VCS in the SBS group was about 45% lower than that observed in the control group. VCS in the cases was reduced across the spatial-frequency profile, but deficits were largest at the mid-to-higher spatial frequencies, similar to effects observed in other cases and groups diagnosed with biotoxin-associated illness [135,137]. These data are shown for comparison only and were not statistically analyzed. The critical statistical comparisons for this study were those made between time points, as discussed in the Results section and in the Fig. 2 legend, in which the current study participants served as their own controls.

4. VCS was classified as normal in only Participant 2 of the 19 participants at Time Point 1 using the criteria described above (Table 4).

Neither the questionnaires on medical history and confounding factors, the physical examination, pulmonary function testing, nor the laboratory analyses indicated a possible cause of multiple-system illness other than chronic exposure to the water-damaged indoor environment in any participant. Pulmonary-function test results indicated normal function in 15 participants, and a restrictive condition in four participants (participant #6: FEV-1/FVC×100=103, FVC=74; #12: FEV-1/FVC×100=122, FVC=73; #16: FEV-1/FVC×100=109, FVC=76; #18: FEV-1/FVC×100=103, FVC=75) at Time Point 1. No participants met the criteria for an obstructive condition. Leptin levels, measured at Time Point 1 in all participants from Buildings 2–5, were above the normal range in all four men and in 10 of 12 female participants (Table 4). Group-mean leptin level was 39.4 ng/ml serum. MSH levels, also measured at Time Point 1 in all participants from Buildings 2–5, were far below the normal range in all participants other than one female who was at the lower end of the normal range (Table 4). The group-mean MSH level was 12.5 pg/ml serum.

3.4. Time Point 2 assessment

Strong recovery was reported by all participants at Time Point 2 following 2 weeks of CSM therapy. The group-mean number of symptoms dropped to 1.6 (Table 4), a statistically significant difference from Time Point 1 [$t(18)=17.10$, $p<0.001$, all symptom p-values are Bonferroni corrected]. Symptom prevalence was near that of controls for most symptoms (Table 2). VCS was classified as normal in 13 of 19 participants (Table 4). The robust recovery in VCS (Fig. 2) was confirmed in group analyses by significantly higher scores than at Time Point 1 [$F(1,18)=72.37$, $p<0.001$, all VCS p-values are Bonferroni corrected]. All VCS analyses indicated a significant effect of spatial frequency, essentially confirming the validity of the VCS test procedure. The significant group×spatial frequency interaction [$F(1,18)=16.50$, $p<0.001$] indicated that VCS had been depressed at some spatial frequencies more than at others, and larger differences at the mid- to higher-spatial frequencies is apparent in Fig. 2. Leptin levels at Time Point 2, only available from eight participants, showed a decrease in six participants (Table 4), but only one participant was in the normal range. After Bonferroni adjustment, group-mean leptin concentration was not significantly lower than at Time Point 1 [$t(7)=2.52$, $p=0.238$].

3.5. Time Point 3 assessment

Recovery continued at Time Point 3 as participants avoided exposure while no longer on CSM therapy. The group-mean number of symptoms was 0.8 (Table 4), a level

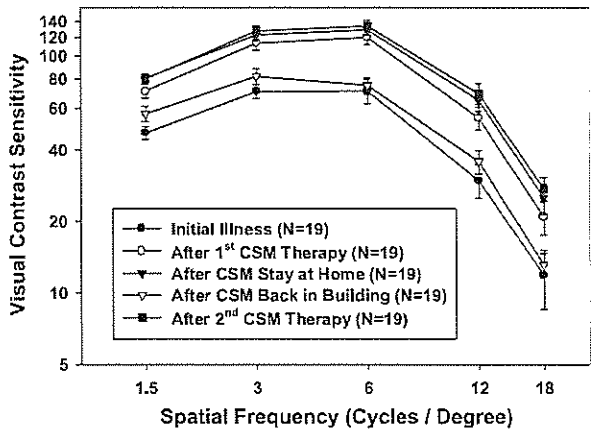

Fig. 2. Group-mean VCS scores (±S.E.M.) for SBS cases at Time Points 1–5. VCS at Time Point 1, when SBS was diagnosed, was significantly below that observed at Time Point 2, after 2 weeks of CSM therapy. VCS remained elevated at Time Point 3, after at least 1 week without CSM therapy while avoiding exposure to the WDBs in which the cases worked. Following assessments at Time Point 3, all cases returned to work in the WDBs without CSM therapy. All cases reported reacquisition of illness within 1–7 days of returning to work, and VCS at Time Point 4 was significantly below that observed at Time Point 3, and statistically equivalent to that measured at Time Point 1. Following another 2-week interval of CSM therapy, VCS at Time Point 5 was again elevated, showing statistical equivalence to that observed at Time Points 2 and 3, but a significant increase relative to Time Points 1 and 4. The normal peak in the VCS function at 6 cpd was apparent at Time Points 2, 3, and 5 when symptom prevalence was low, but was absent at Time Points 2 and 4 when symptom prevalence was high.

significantly lower than at Time Point 1 [$t(18)=16.67$, $p<0.001$], and Time Point 2 [$t(18)=3.75$, $p<0.015$]. Symptom prevalence remained stable or declined further for all but two symptoms (Table 2). VCS showed further improvement (Fig. 2) to a level significantly higher than at Time Points 1 [$F(1,18)=121.55$, $p<0.001$] and 2 [$F(1,18)=15.52$, $p=0.010$]. VCS was classified as normal in 14 of 19 participants (Table 4). Leptin levels were not measured at Time Point 3.

3.6. Time Point 4 assessment

All participants returned to their office buildings without CSM therapy following Time Point 3. After 1–7 days of reexposure (Table 3), all participants reported relapse when assessed at Time Point 4. The group-mean number of symptoms increased to 9.1 (Table 4), a level significantly higher than that at Time Point 3 [$t(18)=16.91$, $p<0.001$], but significantly lower than that at Time Point 1 [$t(18)=6.68$, $p<0.001$]. The prevalence of each symptom was greater at Time Point 4 than at Time Point 3 (Table 2). Group-mean VCS scores (Fig. 2) declined to a level significantly below that measured at Time Point 3 [$F(1,18)=101.20$, $p<0.001$], but similar to that observed at Time Point 1 [$F(1,18)=2.72$, $p=0.160$]. VCS was classified as normal in only 2 of 19 participants; only participants 11 and 17 had normal VCS (Table 4). Leptin levels at Time Point 4, only available from seven participants for which it was measured at Time Point 1, showed an increase in all seven participants (Table 4), none of whom were in the normal range. Group-mean leptin concentration was significantly higher than at Time Point 2 [$t(6)=4.81$, $p=0.018$].

3.7. Time Point 5 assessment

Following relapse during the reexposure phase of the study, participants again underwent CSM therapy for 2 weeks. Health assessments at Time Point 5 indicated substantial improvement. The group-mean number of symptoms reported decreased to 1.2, a level significantly lower than that at Time Point 4 [$t(18)=19.83$, $p<0.001$], but similar to that reported at Time Points 2 [$t(18)=1.92$, $p=0.704$] and 3 [$t(18)=1.24$, $p>0.999$]. VCS scores showed a similar pattern; VCS scores at Time Point 5 were significantly higher than at Time Point 4 [$F(1,18)=161.72$, $p<0.001$], but similar to that observed at Time Point 3 [$F(1,18)=3.12$, $p=0.940$]. VCS was classified as normal in 15 of 19 participants (Table 4). Group-mean Snellen visual-acuity scores (left eye=20:20.5; right eye=20:22.2) did not differ significantly in any pair of study time point comparison. Pulmonary-function test results from the four participants that had a restrictive conditions at Time Point 1 were within normal limits at Time Point 5. Pulmonary function parameters showed improvement in all but one participant, although no pulmonary medications were used by any participant during the study. The group-mean changes in FVC (Time Point 1=86.6, Time Point 5=94.2) and FEV-1 (Time Point 1=86.6, Time Point 5=96.3), respectively, were statistically significant [$t(11)=3.01$, $p=0.012$; $t(11)=3.49$, $p=0.005$], but the change in FEV-1/FVC$\times$100 (Time Point 1=100.5, Time Point 5=102.8) was not significant [$t(11)=0.80$, $p=0.441$]. Leptin levels (Table 4) decreased between Time Points 1 and 5 in 15 of 16 participants, but was in the normal range for only two participants at Time Point 5. The decrease in group-mean leptin level (Time Point 1=39.4; Time Point 5=24.0) was statistically significant [$t(15)=4.40$, $p=0.003$], as was the decrease between Time Points 4 and 5 [$t(7)=6.32$, $p=0.002$]. MSH data were available at Time Point 5 from 10 participants for whom MSH was measured at Time Point 1 (Table 4). MSH levels increased in all 10 participants, for whom group-mean MSH more than doubled to 28 from 12.5 pg/ml serum at Time Point 1, a statistically significant difference [$t(9)=3.66$, $p=0.005$]. Only three of the participants, however, had returned to the normal range.

4. Discussion

The general study hypothesis, that SBS is associated with chronic exposure to WDBs, was supported by the study results. Specifically, the confirmatory hypotheses, that the group-mean number of symptoms and VCS scores would show statistically significant improvement at Time Points 2, 3, and 5 relative to Time Points 1 and 4, were confirmed. Medical assessments at Time Points 1 and 4 followed exposure to the WDBs during periods when participants were not on CSM therapy. The results indicated a multiple-system syndrome characterized by a high group-mean number of symptoms and large VCS deficits, relative to the participants' own scores at Time Points 2, 3, and 5. The results from assessments at Time Points 2 and 5 indicated that CSM therapy led to rapid symptom resolution and VCS recovery, as previously seen in the biotoxin-associated illness, PEAS [135,137]. The continued low level of symptoms and high VCS scores observed at Time Point 3, following a period of exposure avoidance without CSM therapy, indicated that relapse did not occur without reexposure to the WDBs. Reexposure to the study buildings without CSM therapy for 1–7 days resulted in the symptom recrudescence and the decline in VCS scores observed at Time Point 4.

Critical components of the experimental design were the following: (1) the exclusion of potential study participants due to alternative causes of illness and potentially confounding factors; (2) oral administration of a standard set of questions by a single, highly trained health researcher to help eliminate the potential for bias in self-administered questionnaires; (3) the use of VCS as an objective indicator of function in a neurologic system; (4) assessments at five time points, thereby allowing participants to serve as their own controls; (5) the interventions of CSM therapy and

removal from exposure that enabled the researchers to record data indicating a dramatic improvement in health status; and (6) reexposure to the WDBs following recovery that enabled the prospective demonstration of illness reacquisition. The use of these design components avoided some of the limitations intrinsic to many previous studies, thereby enabling the study to produce evidence that indicated exposure to the WDBs was strongly associated with illness.

Several study limitations, however, reduced confidence in the conclusion drawn above. First, the inability to accurately characterize chronic, airborne exposure to the complex mixture of microbes in the indoor environment of WDBs prevented the association of illness causation with any particular genera or species. Only long-term air monitoring could accurately characterize chronic exposure because the rates at which spores or cells and particles are emitted to air vary with the factors mentioned above [111]. Differences between study buildings in the predominant microbes detected prior to Time Point 1 (Table 1) indicated that a variety of organisms may be etiologic agents. Second, the inability to accurately characterize the complex mixture of mycotoxins, endotoxins, and antigens in human tissues that may result from chronic exposure to WDBs prevented the association of illness with any particular bioactive substance or mixture. Animal studies are needed to test additional hypotheses concerning microbial, toxin, and antigen causation of illness. Third, the prevalence of SBS among study-building occupants could not be estimated because neither was the entire population assessed, nor was the population randomly sampled. Index cases from each building recruited the potential study participants. Workers who volunteered for screening may have been more affected by exposure than those who did not volunteer. Screening indicated that not all volunteers met the symptom criteria for SBS (Table 1). Preliminary results from another study indicated that polymorphisms in the human leukocyte antigen (HLA) DR and DQ genes on chromosome 6, as measured by polymerase chain reaction, may have influenced individual susceptibility to chronic illness following exposure to WDBs [136]. HLA, the most polymorphic portion of the genome, codes for proteins involved in antigen presentation to T-cells [133]. The inability to sensitize T-cells by effective presentation of antigens or toxins bound to protein may render elimination or neutralization processes ineffective. An important consequence of individual differences in protective processes is that dose–response functions would not accurately predict illness in all individuals. Individuals with effective protective processes might not develop illness, whereas individuals with ineffective protective processes could remain chronically ill even after removal from exposure because of retention of antigens and toxins. Fourth, the physician who conducted the assessments was not blinded with respect to the assessment Time Points. However, the critical endpoints were objective measurements, and the questionnaires were administered using a standard procedure. Fifth, the study participants were aware of when they took CSM. It is possible that participants felt better simply because they knew they were receiving medication. However, the previous double-blinded, placebo-controlled, crossover clinical trial demonstrated that biotoxin-associated illness resolved only during CSM therapy [135]. Further human and animal research is needed to verify the current study results and to address additional hypotheses concerning illness causation and susceptibility factors.

The SBS cases were treated with CSM due to results seen in our previous studies of biotoxin-associated illness [135,137], and results from case reports and animal studies. CSM, a polymer too large for gastrointestinal absorption, was the first treatment approved by the U.S. Food and Drug Administration (FDA) for hypercholesterolemia. The theoretical basis for CSM use in the SBS cases was that toxin elimination rates can be enhanced through anion exchange or other binding of CSM with toxins. Binding causes the toxins to be eliminated, thereby interrupting the enterohepatic recirculation of toxins with bile and systemic recirculation. CSM has successfully enhanced the elimination rates of kepone [16,36], DDE [116], other organochlorine pesticides [128], polychlorinated biphenyl compounds [19], *Clostridium difficile* toxin [103,113], *Escherichia coli* and *Vibrio cholera* toxins [17,126], one or more cytotoxins from at least one unidentified gastrointestinal microorganism [4,82], the fungal mycotoxins ochratoxin A [39,96,97], fumonisin B1 [155], and zearalenone [151], the cyanobacterial toxin microcystin LR [44], and a toxin from the Chinese herbal product Jin Bu Huan [26]. A pharmacologic study demonstrated that the plasma half-life of M1, the active metabolite of Arava (leflunomide), a pyrimidine synthesis inhibitor FDA approved for treatment of rheumatoid arthritis, was lowered from >1 week to approximately 1 day following CSM administration due to CSM binding of M1 from bile. These results indicated that biliary recycling with systemic recirculation was a major contributor to the long half-life of M1 [8], as it also may be for toxins and antigens acquired through exposure to water-damaged indoor environments. This reasoning is consistent with the improvement seen in VCS and symptom scores at Time Points 2 and 5. CSM treatment likely reduced the period of illness in the SBS cases by months or longer, as the cases reported little or no symptom abatement during previous periods without exposure to the buildings. Only a few patients experienced treatment side effects, constipation or acid reflux, which were easily controlled with standard therapies. These results indicated that CSM therapy is a highly effective therapy for relieving multiple-system and nonspecific symptoms among occupants of water-damaged indoor environments.

The change in group-average VCS scores during the time series provided an objective indication of health status as robust as that from the subjective symptom scores. VCS

testing also provided a sensitive and specific indicator for individual assessment. Using the criteria described above, VCS was normal in only one participant at Time Point 1 and in only two participants at Time Point 4. These results contrasted sharply with the observations of 13, 14, and 15 normal scores at Time Points 2, 3, and 5, respectively. Assuming that all participants were ill at Time Point 1 and well at Time Point 5, sensitivity of the VCS test at Time Point 1 was 18 hits/19 ill participants×100=95%; specificity at Time Point 5 was 15 correct rejections/19 well participants×100=79%. VCS showed rapid alterations in all participants, perhaps due to changes in blood flow rates in the retina and/or brain. Proinflammatory cytokines constricted microvasculature and reduced blood flow rates in rat and sheep brain and lung [48,63,138,155,157]. Blood flow rates in retinal microvasculature were depressed when VCS was reduced in other biotoxin-associated illnesses (Shoemaker—unpublished data). Enhanced rates of toxin elimination due to CSM therapy may have led to a rapid decrease in proinflammatory cytokine levels, increased blood flow rates in microvasculature, and rapid VCS recovery. However, because proinflammatory cytokine levels and blood flow rates were not measured in the current study, these possibilities remain speculative. In any case, the results strongly supported the position that VCS testing provides a simple, noninvasive, and inexpensive tool with high diagnostic value in biotoxin-associated illness when used with thorough screening for potentially confounding factors.

Confirmation of the hypotheses concerning leptin and MSH indicated that they may play important roles in the course of illness. Although leptin and MSH levels moved in the expected directions on a group-mean basis and in most individuals following CSM therapy, they did not return to the normal range in most participants. Analyses for leptin and MSH, respectively, indicate sensitivity=88% and 94% at Time Point 1, and specificity=25% and 30% at Time Point 5. It is unclear whether leptin and MSH levels would eventually reach normality or remain abnormal due to persistent or permanent damage.

Leptin, 143 amino acid, 16 kDa adipocyte hormone, has been reported to affect regulation of body weight, hypothalamic activity, and response to feeding [142]. A pharmacokinetic characterization of leptin showed transport across the blood–brain barrier, and binding to the long isoform of its receptor, which is closely related to a primordial gp-130 class 1 cytokine receptor [64], that is present in the arcuate nucleus, paraventricular nucleus, and ventromedial nucleus of the hypothalamus [90]. The report that leptin deficiency was accompanied by increased susceptibility to endotoxin and decreased induction of anti-inflammatory cytokines [64] indicated a physiologic link between leptin and MSH production that could be adversely affected by a proinflammatory cytokine effect on the leptin receptors in the hypothalamus. Normally, after leptin interacts with its receptor, a Janus tyrosine kinase (JAK)-STAT signaling cascade activates transcription of proopiomelanocortin [106] with cleavage of the prohormone causing MSH release [34]. The observation of elevated leptin levels and MSH deficiency suggested the development of a central receptor resistance, perhaps similar to the resistance created by disruption of tyrosine kinase in Type II diabetes, caused by proinflammatory cytokine responses [43].

MSH, a 13 amino acid peptide derived from post-translational processing of proopiomelanocortin, levels were reported to be reduced in chronic, fatiguing illnesses with evidence of increased levels of proinflammatory cytokines [22] An investigation into the role of MSH in the current study was indicated by (1) the ability of MSH to modulate production and action of proinflammatory cytokines in periphery and central nervous system by down-regulation of nuclear factor kappa B [86]: (2) its role in modulation of disorders in which infection and inflammation coexist [25]; and (3) evidence that administration of MSH reduces levels of the proinflammatory cytokines, tumor necrosis factor alpha and interleukin 1, in endotoxin-stimulated patients with sepsis syndrome [24]. Further evidence of MSH deficiency in illnesses with an inflammatory basis, including the response to biological toxins, was indicated by an "anticytokine", neuroimmunomodulatory effect mediated through MSH receptors, MSH effects on macrophages and granulocytes, and descending neural pathways originating from MSH-activated neurons in the central nervous system [23,105]. MSH has been reported to exert regulatory effect on pituitary function by controlling growth hormone release [14], gonadotrophin release [127], and possibly vasopressin release [130]. Behavioral functions reported to be influenced by MSH include verbal memory, pain perception [7], attention, and goal-motivated behavior [160]. Additional evidence indicated that MSH has the capacity to affect cerebral protein synthesis, RNA synthesis, and protein phosphorylation, thereby potentially altering the capability of an organism to both evaluate information and interact effectively with its environment [12]. Additional research is needed to clarify the modes of action by which toxins and antigens may elevate leptin and reduce MSH levels, and the roles of these hormonal alterations in producing the signs and symptoms associated with illness among SBS patients.

In conclusion, the study results supported the hypothesis that chronic exposure to the water-damaged environments of the study buildings was associated with SBS. The use of a powerful, modified ABAB experimental design and objective endpoints added strength to this conclusion. The use of double-blind and placebo-control components would further strengthen future studies. Although the study results indicated that chronic exposure to WDBs is a human health risk, the results were not sufficient to quantitatively assess the risk. Animal studies are needed to better characterize the causative agents, interactions

between agents in complex mixtures, and their modes of action.

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